EXHIBIT E

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

---------- X	**MDL No. 1358 (SAS)**
In Re: Methyl Tertiary Butyl Ether ("MTBE") Products Liability Litigation	**Master File C.A. No. 1:00-1898 (SAS)**
This document relates to:	
City of New York v. Amerada Hess Corp., et al., Hess Corp., et al.	**No: 04 CV 3417**
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EXPERT REPORT OF DOUGLAS McGREGOR, Ph.D

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Signature

March 9, 2009
Date

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Opinions on the Toxicology of Methyl *tertiary*-Butyl Ether (MTBE)

9 March 2009

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* page numbering can only be approximate, because of the page size difference Europe-USA.

Introduction

I have been asked by Lyondell Chemical Company and Equistar Chemicals LP to review and comment upon the report from Dr. Burns that has been presented by the plaintiffs in the lawsuit filed by the City of New York versus Amerada Hess Corp. et al., 04 Civ 3417. In addition or conjunction therewith, I have been asked for my own opinions with respect to the toxicological profile of MTBE. My comments and opinions, along with the foundation and basis thereof, are set forth below and in my review of MTBE toxicology as published in 2006 in a peer-reviewed journal, a copy of which is attached as Exhibit A to this report and incorporated herein.

Qualifications

My education, experience and training are set forth in detail in the curriculum vitae attached as Exhibit B to this report. In brief, my qualifications to offer opinions on the toxicology pertaining to MTBE include:

- About 25 years of laboratory work, 20 of which were in toxicology using a wide variety of *in vivo* and *in vitro* techniques, either directly or as a study supervisor (SD, PI); this included mutagenicity testing of TBA (published in 1988, my first contact with MTBE) and was followed by
- 11 years employment in carcinogen hazard and risk evaluation in the International Agency for Research on Cancer (IARC), an agency of the World Health Organisation (WHO); this included participation in the *IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans* meeting (1998) at which MTBE was evaluated; followed by
- 9 years occupation as an independent consultant in toxicology with continued involvement in hazard and risk evaluation for non-commercial, national and international organisations –
    - International Programme on Chemical Safety, IPCS;
    - European Food Safety Authority, EFSA;
    - l'Institut National de Recherche et de Sécurité (INRS), France;
    - l'Institut de Recherche Robert-Sauvé en Santé et en Sécurité du Travail (IRSST), Québec, Canada;
    - Republic of Ireland at the Committee for Veterinary Products (CVMP) of the European Medicines Authority (EMEA); and in addition,
    - I have also consulted in toxicology to several commercial enterprises in the petrochemical, metallurgic, pesticide, food and medicinal product areas.
- Several peer-reviewed publications on modes of action of chemical carcinogens;
- Peer-reviewed publications on the toxicology of MTBE and ETBE.

Compensation

My compensation is US$250.00/hr for preparation time; US$125.00/hr for travel time; and US$350.00/hr for court time.

Prior Testimony

I was deposed and I provided trial testimony in the case of South *Tahoe Public Utility District versus Arco et al.* in 2001 and 2002.

I provided testimony in the hearing *Aldéric Morissette and Ville de Québec* in 2008.

I was deposed in the following cases, but did not provide trial testimony: (1) San Jose IBM Workers Litigation in 2003. (2) *D.J.Nelson versus Atlantic Richfield et al.* in 2006 (3) *Suffolk County Water Authority and United Water New York, Inc. versus Amerada Hess et al,* in 2008.

Opinions

The opinions to which I expect to testify to a reasonable degree of scientific certainty are as follows:

1. The existence of "key symptoms" initially reported as associated with MTBE exposure has not been verified;

2. Adverse responses in people exposed to MTBE are highly unlikely to occur at likely or exaggerated exposure levels;

3. Absorbed MTBE is rapidly metabolised primarily to *tertiary*-butyl alcohol (TBA) and formaldehyde and eliminated by volunteers and rodents; formaldehyde (which is also a product of normal metabolism) is very rapidly removed by further metabolism; the kinetics of MTBE strongly suggest that toxicity data obtained following inhalation can be extrapolated to oral exposure;

4. Very high dose levels are required to produce neurological effects in rodents; these effects are reversible within a short time and are not associated with histopathogical lesions;

5. The weight of evidence from rodent and rabbit studies for toxicity to reproduction (fertility or development) is clearly in favour of MTBE not being a human reproductive toxicant at likely or exaggerated doses;

6. The weight of evidence is clearly in favour of both MTBE and TBA, the more long-lived of its primary metabolites, being non-mutagenic;

7. Because of (6) any carcinogenesis of MTBE would involve a non-mutagenic mode of action;

8. While there are reports of increased incidences of various types of tumours in rodent studies with MTBE, TBA and methanol (included as a model, endogenous precursor of formaldehyde without the simultaneous presence of TBA), these are uncertain, inconsistent or, where there is some degree of replication, without human relevance. The weight of evidence is clearly in favour of MTBE not being a human carcinogen at likely or exaggerated doses.

9. Suggestions that have been made for MTBE being damaging in other respects are based on poor evidence.

10. To summarise, in my opinion, MTBE in drinking water has never caused anyone harm and the sufficient and extensive toxicology database strongly suggests it never will cause anyone harm because it shows that MTBE has low toxicity and, should water supplies become badly contaminated, taste and odour will cause self-limitation of intake.

I arrived at the above stated conclusions on the basis of the following general principles and MTBE-specific information to be described later in this document.

General Principles

1. There are identifiable features that distinguish scientific inquiry from other methods of developing knowledge. Scientific researchers propose specific hypotheses and design experiments that test these predictions, but experiments do not necessarily give the same or even similar results when repeated. Consequently, it is a part of scientific method that these experiments are repeated – and the results confirmed - in order to make increasingly dependable predictions of future results. In toxicology, results should be both reproducible and dose-dependent. If these criteria are not met then the hypothesis should be rejected and revised. This ideal situation may not always be met, however, and an evaluation has to be made on the basis of a single experiment. Whenever this is done, any conclusion reached is weaker than when there has been an opportunity to repeat an experiment and collect corroborating information.

2. Commonly used terms that are used in toxicology must be defined, because they may be used differently by other witnesses in this case.

a. "Dose" (OECD, 2003): Total amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population. A related term is "Exposure," which is the concentration (c) or amount of a particular agent that reaches a target organism, system, or (sub)population in a specific frequency for a defined duration (t), i.e., exposure = c x t. "Concentration" can be a quantity of an agent per unit mass or quantity per unit volume.

b. "Hazard" (OECD, 2003): Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population is exposed to that agent.

c. "Risk" (OECD, 2003): The probability of an adverse effect in an organism, system, or (sub)population caused under specified circumstances by exposure to an agent.

3. All substances can be identified as hazards at some dose level. This is a principle that has been fundamental to toxicology for centuries. Hazard is a term that can be applied to any agent if it is present in high enough doses, whereas risk is applied to particular dose or exposure scenarios and may be described as negiligible, moderate, high, severe, etc.

4. Experiments can be used to identify a No Observed Adverse Effect Level (NOAEL), or some other value considered by regulators as being of negligible toxicity within the experiment. The Benchmark Dose (BMD) procedure, which is being used increasingly, provides such an alternative "reference point" (RP) or "point of departure" (PoD). The BMD is based on a mathematical model being fitted to the experimental data within the observable range and estimates the dose that causes a low but measurable response (the Benchmark Response, BMR), typically chosen at a 5 or 10% incidence above the control (U.S. EPA 1995). Such incidence levels would normally be at the limit of statistical significance in toxicological experiments conducted according to internationally agreed guidelines, which take account of both statistical power and animal welfare issues. The BMD lower limit (BMDL) refers to the corresponding lower limits of a one-sided 95% confidence interval on the BMD. Using the lower bound takes into account the uncertainty inherent in a given experiment, and assures (with 95% confidence) that the chosen BMR is not exceeded. It is this more stringent value that provides the RP or PoD, which may be used as an

alternative to the NOAEL. Underlying the use of such terms is the principle that, although there may be negligible effects at this dose level in the experiment performed, should the experiment be repeated, even in the same species, the numerical value obtained may be different, so it cannot be regarded as synonymous with a safe dose.

5. Having obtained an NOAEL, RP or PoD, uncertainty factors (Renwick, 1993; WHO, 1994; Renwick and Lazarus, 1998) are applied that allow for:

a. uncertainty in the initial NOAEL, RP or PoD determination. This is a standard 10-fold that contains factors for differences in toxicokinetics (metabolism, etc.) and toxicodynamics (tissue response in terms of damage).

b. uncertainty in extrapolating from one species (e.g., rat or mouse) to another (e.g., man). This also is a standard 10-fold.

Thus, species differences and human variability in the basic process of toxicokinetics and toxicodynamics are inherent in the use of data from studies in animals for human risk assessment. An overall factor of 100-fold is usually used to allow for these uncertainties (a and b, above) in the risk assessment of non-genotoxic substances that may or may not also be carcinogenic. Similar uncertainties would be applicable to substances that are both genotoxic and carcinogenic. In addition, however, there are other uncertainties that should be applied specifically for substances that are both genotoxic and carcinogenic and where genotoxicity is a likely mode of action (MOA), because of the inter-individual human variability in cell cycle control and DNA repair, which influence the carcinogenic process (Dorne and Renwick, 2005; EFSA, 2005).

Before introducing factors for such additional uncertainties, a genotoxic MOA should have been proposed and supported by evidence indicating that this MOA is at least reasonable.

Uncertainty factors may also be applicable if an LOAEL is used, rather than an NOAEL, if the NOAEL is from a short-term study, rather than a chronic study and if the database is considered to be incomplete in some way. It is to be understood, however, that the application of uncertainty factors is not some mechanical process, but done after careful consideration of the data.

c. The risk assessors, having completed their task, present the data and their conclusions to the risk managers, who may modify the uncertainty factors in a way they see as prudent. The "precautionary principle" is a risk manager's tool and should not also be used by the risk assessors. If the risk assessor was to apply this principle then it will likely be applied twice, so that precaution becomes overly protective and without a sound basis.

Application of these General Principles to MTBE.

1. In the case of MTBE, an overall factor of 100-fold on the NOAEL, RP or PoD would be a reasonable health-based risk assessment conclusion if the basis was carcinogenicity because the database covers all of the endpoints that are normally requested and the tumours that have been reported at increased incidence are:

- of no human relevance although reproducible (Leydig cell adenomas of the rat testis);
- of no human relevance (renal tubule cell tumours specific to male rats); or,

- of uncertain biological status as will be discussed below ("lymphohaematological neoplasms" in female rats in the absence of any report of toxicity in the relevant tissues, hepatic adenomas in female mice at the single very high concentration of 8000 ppm in air, but not at 3000 ppm or lower, and thyroid follicular cell tumours in female mice exposed to TBA, the primary, more persistent metabolite of MTBE).

Although some significant responses have been reported in some studies of genetic toxicity, the overwhelming majority of studies – including all studies *in vivo* – did not show any significant effects in response to MTBE or TBA exposure. Consequently, the genetic toxicity and mutagenicity database does not provide a basis for proposing a genotoxic or mutagenic MOA for any of these reports of higher incidence of tumours. Furthermore, MTBE has never been classified as a genotoxin or a mutagen by national regulators. The increased incidences of these tumours do not, therefore, encourage the application by the risk assessor of safety factors in addition to the 100-fold that would be applied to any endpoint not involving mutagenicity. However, it is not carcinogenicity that provides the Reference Point, Point of Departure or overall NOAEL; this is provided by clinical signs of toxicity in adult rats exposed to MTBE in a two-year inhalation study for chronic toxicity and carcinogenicity in rats (Chun et al., 1992). Similar observations of clinical signs of toxicity were recorded in adult rats during a two-generation study of reproduction (Bevan et al., 1997a). The NOAEL in both of these studies is 400 ppm, equivalent to approximately 150 mg/kg body weight/day in the reproduction study and approximately 140 mg/kg body weight/day in the two-year study. This value therefore encompasses the apparent LOAEL of 400 mg/kg body weight/day in the recent, but less reliable study of testicular toxicity (Li et al., 2008), to which an additional uncertainty factor of up to 10-fold should be applied because 400 mg/kg body weight/day is not an NOAEL but an LOAEL. Thus all three studies provide the same allowable daily intake of MTBE that is predicted not to produce harm.

2. Currently, the most recent authoritative comprehensive review of the data on MTBE is that undertaken by the European Union (EU, 2002). Finland was the rapporteur Member State and the document was discussed in committee throughout its evolution by representatives of all the Member States who were members of the EU at that time. Authoritative documents have also been produced by the Centers for Disease Control (CDC, 1996; WHO/IPCS, 1998) and by Canada, but that work was undertaken much earlier and could not take account of more recent studies (Canada, 1992). There have been other authoritative reviews, but these are more specialised (e.g., IARC, 1999; NTP, 2000).

The EU conclusions on the quantitative aspects of chronic effects were as follows.

a. Inhalation NOAEC of 1450 mg/m^3, based upon a rat carcinogenicity study in which the incidence of Leydig cell tumours was significantly increased at 11,000 mg/m^3 (Chun et al., 1992; Bird et al., 1997). The figure 1450 mg/m^3 resulted in a dose to the rats of approximately 310 mg/kg body weight/day over two years.

b. In searching for a suitable dose on which to assess risk following oral administration, the EU could not completely discard the LOAEL of 250 mg/kg from the study conducted by Belpoggi et al. (1995). Although it was decided to use this study for the derivation of a margin of safety due to lack of other oral carcinogenicity data, the reporting and overall conduct of this study was challenged, and they clearly

did not have complete confidence in the results. These doubts have increased in the intervening years, as will be mentioned below.

This conclusion differs from the earlier conclusion of the CDC (CDC, 1998), in which it is stated (p 115), "An MRL was not derived for chronic-duration oral exposure to MTBE because in the only chronic oral study (Belpoggi et al. 1995), increased mortality occurred in female rats at the lowest dose tested (250 mg/kg/day). Furthermore, the dose of 250 mg/kg/day was associated with dysplastic proliferation of lymphoreticular tissues and an increased incidence of lymphoma and leukemia in female rats." While the first reason (reduced survival) is correct for female rats, the situation was reversed for male rats, in which survival in the high dose group was better than in the control group. The second reason (lymphoreticular pathology) is at least questionable because of the now widely-expressed doubts regarding the biological significance of the lymphohaematopoietic neoplasm incidence.

These three different views will now be used to arrive at Maximum Contaminant Level estimates, using the uncertainty considerations described above and common default values for human body weight (70 kg) and daily water consumption (2L). Other default values for biological parameters were as applied by the US EPA (1988).

1. NOAEL of 400 ppm equivalent to approximately 340 mg/kg body weight/day based on clinical signs of toxicity in rats at 3000 ppm equivalent to approximately 2550 mg/kg body weight/day (Chun et al., 1992; Bevan et al., 1997a) and the application of a basic uncertainty factor of 100-fold.

3400 µg/kg body weight/day = 238,000 µg/person/day.

Therefore the concentration of MTBE in drinking water should not exceed 238,000/2 = 119,000 µg/L.

A risk manager may wish to take public opinion into account and apply a 10-fold uncertainty factor for these concerns. This would reduce the maximum concentration of MTBE in drinking water to 11,900 µg/L. It should be pointed out, however, that there is already extra caution (2-fold) built into the risk assessment value I arrived at: Daughtrey et al. (1997) found no signs of CNS effects or clinical signs of toxicity in rats exposed by inhalation to 800 ppm MTBE for 6h.

2. The EU's LOAEL of 250 mg/kg body weight/day, based on reduced body weight gain in female rats and lymphoreticular pathology at this, the lowest dose level, in a lifetime gavage administration study of toxicity in rats (Belpoggi et al., 1995, 1997, 1998) and the application of a basic uncertainty factor of 100-fold and an additional 10-fold because the dose was an LOAEL, not an NOAEL.

250 µg/kg body weight/day = 17,000 µg/person/day.

Therefore the concentration of MTBE in drinking water should not exceed 17,500/2 = 8,750 µg/L.

3. CDC (at least in 1996) was not prepared to use the Belpoggi et al. study because there was no NOAEL.

Although the first two estimates might not carry a human health risk, it is clear that such concentrations of MTBE would not be tolerated for other reasons, particularly: the taste and odour of the chemical. This is a topic outside of my expertise. In Europe the threshold is accepted as 15 µg/L (15 ppb) (EU 2002).

Discussion

The remainder of this document is largely a response to the opinions expressed by Kathleen Burns, PhD regarding the toxicology of MTBE.

Fundamentally, the position taken by Dr. Burns on the toxicology of MTBE is as follows:

"Based on substantial scientific evidence, MTBE in drinking water is likely to pose health hazards2 to some members of the public. MTBE caused cancer in animal models that are relied upon by the US government to predict cancer in humans. MTBE damages genetic material and caused other serious health problems in multiple species that the US government relies upon to evaluate the potential for birth defects and other types of damage. There is no credible or proven "safe" level of MTBE exposure and there is substantial and evidence that no safe level exists."

A superficial reading of Dr. Burns' opinions would suggest that they are fully supported by the scientific evidence. In my opinion, however, a more informed reading of Dr. Burns' text, based on knowledge of the scientific evidence that is available, does not support the conclusions and opinions expressed. Furthermore, it is remarkable that, throughout Dr. Burns' long opinion statement, there is hardly a mention of a dose level and no attempt to distinguish hazard from risk. Both dose and risk are fundamental to toxicological evaluation and their absence from Dr. Burns' statement undermines any pretence of expert authority.

The statement by Dr Burns (p5) that there is "no credible or proven 'safe' level of MTBE exposure and there is substantial and [*sic*] evidence that no safe level exists," so that only an undetectable level would be safe are presumably based on the conclusions that MTBE is a carcinogen and its mode of carcinogenic action is mutagenicity. Even if it were arguable that MTBE is carcinogenic, which I do not concede, the overwhelming weight and strength of evidence is that MTBE is not mutagenic.

In the following paragraphs I shall rebut the opinions expressed by Dr. Burns on the toxicology of MTBE and in that process I shall, inevitably, state my own. In addition, I shall state my opinion regarding the suitability of the MTBE toxicology data-base for assessing risks, if any, posed by MTBE as a contaminant of drinking water.

I should like to present as an exhibit my review of MTBE toxicology as published in a peer-reviewed journal: McGregor, D. (2006) Methyl *tertiary*-Butyl Ether: Studies for Potential Human Health Hazards. *Crit.Rev.Toxicol.*, **36**, 319-358. This publication contains many of my opinions on the hazard and risk assessment of MTBE, but as it is now three years since it was published and new data have become available, it will be necessary now to take them into account.

To begin, the behaviour of MTBE and its metabolites within the body need to be described. This information does not, by itself, lead to any conclusions regarding the toxicity of the chemicals, but it is necessary for an understanding of MTBE toxicology.

1. Metabolism and Kinetics

The usual way of describing how long a chemical remains in the body is to state how long it takes for the starting concentration of a chemical to be reduced to half of that concentration. This value is called the $T_{½}$ and its units may be seconds, minutes

or hours; less commonly, larger units also may be used (e.g., for dioxins). Often, more than one $T_{½}$ value is quoted even within the same study. This may come about because the value depends on measurement of concentrations in blood and a chemical may have distributed to different tissues of the body from which it can be released back into the blood at different rates or, very important, it may have been converted (metabolised) to another chemical altogether. This can lead to elimination occurring in several "phases," each with its own $T_{½}$ value. Distribution, in its turn, can vary according to the dose route (inhalation, oral, dermal). Finally, the measurements made on blood samples are not continuous and the intervals between taking one blood sample and the next often vary between one study and another. This means that the precision of the $T_{½}$ estimate also may vary between one study and another.

Measurements of $T_{½}$ values have been made in several experiments with human volunteers, usually following exposure to MTBE by inhalation. In two studies, however, other routes have been investigated: inhalation and oral (Amberg et al., 1999; Dekant et al., 2001); inhalation, oral and dermal (Prah et al., 2004). In the earlier study, elimination was in 2 phases after inhalation (approximately 1.3h and 2.4h) and in 3 phases after ingestion (approximately 45 min, 1.5h and 4 – 8h). In the Prah et al. study elimination was observed to occur in 3 phases with similar $T_{½}$ values irrespective of the dose route. These were approximately 2-15 min, 1-2h and 5 – 7h. Measurements in the Prah et al study showed that concentrations have returned to background (100 – 1000 times lower than the maximum, starting concentration) after about 25h, again, independently of dose route.

MTBE disappears from blood most prominently as a result of metabolism to TBA, which in its turn is eliminated, largely by metabolism, but at a slower rate. This difference can lead to TBA concentrations being greater than those of MTBE. In fact, in the Prah et al. study the blood concentration of TBA was approximately constant over 24h (by which time TBA concentration in blood was 7 – 10-fold greater than MTBE) and the concentrations of TBA in blood were approximately the same after inhalation and oral dosing (by comparison, concentrations of TBA were approximately 3-fold lower after dermal administration). TBA is eliminated by conjugation with substances made within the body or converted to other degradation products (which, interestingly, are also products of metabolism of some normal constituents of our bodies). Finally, all of these products are voided from the body, mainly in urine. Similar measurements have been made in rats, but because of the more severe limitations on the number and volume of blood samples that can be taken they are less precise than the human studies. Nevertheless, although they are different in absolute terms from the human values, the dose route comparisons made in rats indicate similarities in blood concentrations and rates of elimination following MTBE exposure by the inhalation and oral routes.

To summarise, MTBE is absorbed, metabolised and eliminated in quantitatively and qualitatively very similar ways after human inhalation and human ingestion. TBA is the major and relatively persistent product, which is metabolised and eliminated in quantitatively and qualitatively very similar ways irrespective of whether it was derived from inhaled MTBE or ingested MTBE. This means that, although most toxicity studies of MTBE have been performed using the inhalation route, it can be assumed that the results would be similar had the oral route been used. The dermal route would be predicted to induce even fewer effects, except to the area of skin where a high concentration of MTBE had been applied. This consideration is

concentration in the water of the patients in the earlier study was above "permissible regulatory levels." Participants were informed of the nature of the blood drawing procedure, but apparently not about why their blood was being taken.

The adduct measured (8-hydroxyguanosine) in the 1999 study *is not specific to MTBE* and can be generated at high levels in the absence of known exogenous oxidising agents and as artefacts during DNA extraction and purification. Its concentration was about $4/10^6$ guanine bases in the DNA from the controls and about $10/10^6$ guanine bases in the DNA from the patients. It cannot be concluded that this rather small difference is due to any difference in exposure to MTBE. Also, the hypothesis that oxidative damage to DNA can arise from MTBE exposure is not supported by Chen et al. (2008), described below, who conclude that BTEX (i.e., a mixture of benzene, toluene, ethyl benzene and xylenes), but not MTBE, produces oxidative DNA damage in human lymphocytes. The available evidence strongly suggests that the Vojdani and Brautbar (1999) is flawed and therefore should not be used to arrive at any conclusion regarding the reactivity of MTBE with DNA.

In another drinking water study by this same group (Vojdani et al., 1997) the biological effects they attributed to MTBE and benzene were not assessed over the same time that chemical measurements were made; furthermore, the exposures to MTBE and benzene were not characterised, so the effects they observed cannot be attributed to either chemical. With this demonstration of lack of care in study desgn and execution, one is left to guess what significance can be attributed to any report from this group.

DNA Damage (Genotoxicity)

Zhou et al. (2000) was reviewed in the McGregor (2006) publication, but Dr. Burns has introduced some errors that need to be corrected. First, it is stated (Burns p35 para5) that unscheduled DNA synthesis (UDS) leads to abnormal cell replication. In fact, UDS is an expression of DNA repair that has been activated by damage recognised by specific repair enzymes: the cell is not stimulated to replicate. Furthermore, the experiment was most likely not performed in rats for reason Burns has offered, but because that is where we have most experience.

The same paragraph (Burns, p35, para5) states that the assay was performed in rat liver because this was the tissue where cancer was caused by MTBE in the 1991 Burleigh-Flayer et al study. Again this is not true. The carcinogenicity study referred to was an experiment with mice, not rats. Tumour incidence was increased in the liver of the female mice exposed to 8000 ppm MTBE by inhalation, but not if exposed to 3000 ppm or 400 ppm MTBE. In the companion experiment in rats (Chun et al., 1992), there was no increased incidence of liver tumours in either male or female rats. Thus, there is no correlation between the induction of UDS in liver and the occurrence of liver tumours. The observation has no bearing on the topic at hand.

Perhaps the most important factor not considered by Dr Burns in evaluating the Zhou et al. (2000) study is that the method they used to estimate UDS has been considered invalid for a long time (Venitt (1986). This was clearly stated in the McGregor (2006) review of MTBE.

In a recent publication, Chen et al. (2008) demonstrated that exposure of human lymphocytes for 1h to MTBE at concentrations of 50 μM (lowest concentration tested) increased the level of DNA damage as measured by the comet assay. This study appears to have been better executed than the Tang et al. (discussed in

McGregor, 2006) study (it is interesting that, although both groups of investigators are Chinese, Chen does not even mention Tang). Chen et al. (2008) performed a second *in vitro* experiment similar to the first, except they incubated the lymphocytes with spin-trap agents before adding the MTBE, 200 μM. Spin-trap agents are used in analytical chemistry for the detection of radical species (which are frequently highly reactive substances). This modification had no effect on the comet assay result, from which they concluded that radicals are not involved in generating DNA damage. However, when they incubated the cells with a DNA repair enzyme, DNA-formamidopyridine glycosylase (a DNA repair enzyme that is an N-glycosyl hydrolase with specificity for DNA-containing ring-opened N(7)-methylguanine residues), as well as 200 μM MTBE, there was an increase in the DNA damage score. The main substrate for this enzyme appears to be 8-oxo-guanine, which is probably the most abundant base-oxidation product of DNA, although it also recognises and excises alkylated DNA (Smith et al., 2006). [To attempt to put the Chen et al. result into a human context, compare the effective concentration in the Chen study (50 μM) with that of Prah et al. (2004) who administered 14 male volunteers oral 11 mg MTBE and found the maximum concentration in blood to be 0.17 μM MTBE at 15 min and 0.23 μM TBA at 75 min. Also, Dekant et al. (2001) administered 6 volunteers 15 mg oral MTBE and found the maximum concentration in blood to be 0.69 μM MTBE at 1h and 1.82 μM TBA at 1h].

The results of Chen et al. (2008) suggest MTBE and BTEX, the other compounds they tested, are genotoxins. If so, what is to be concluded about toluene and the xylenes, which are not carcinogenic according to IARC and US NTP? Furthermore, the relevance of the MTBE-associated tumours was clearly questioned by IARC (MTBE, was classified as *Group 3, not classifiable as to its carcinogenicity in humans* on the basis of *inadequate evidence* in humans and *limited evidence* in experimental animals).

4. Carcinogenicity

Chronic toxicity and carcinogenicity studies have been conducted with MTBE and its primary metabolite, TBA, on five occasions.

The studies were:

MTBE inhalation by rats (Chun et al., 1992*, Bird et al., 1997)

MTBE inhalation by mice (Burleigh-Flayer et al., 1992*; Bird et al., 1997)

MTBE orally by gavage by rats (Belpoggi et al., 1995; Belpoggi et al., 1997; Belpoggi etal., 1998; Maltoni et al., 1999)

TBA in drinking water by rats (NTP, 1995*; Cirvello et al., 1995)

TBA in drinking water by mice (NTP, 1995*; Cirvello et al., 1995).

* indicates the original and complete study report; none is available for the MTBE oral gavage experiment in rats.

I have considered all of these and, in addition, I have considered similar studies on formaldehyde, also a metabolite of MTBE, but one which in all probability is never released as such within the body, and methanol. I thought that the behaviour of methanol in such studies could be interesting and perhaps important because it is also metabolised to formaldehyde, but, unlike MTBE, any effects observed are not complicated by the simultaneous release of TBA.

According to Dr. Burns, MTBE caused multiple types of cancer in multiple study species and that it is mutagenic, providing additional evidence that it is a carcinogen. She also believes that MTBE is carcinogenic through a genotoxic mode of action.

The International Agency for Research on Cancer (IARC), in its Monographs Programme on the Evaluation of Carcinogenic Risks to Humans (1999a) concluded that MTBE "is *not classifiable as to its carcinogenicity to humans (Group 3)*. Group 3 is 4th in the series Group 1, 2A, 2B, 3 and 4. There is only one agent (the nylon monomer, caprolactam) in Group 4 out of well in excess of 900 evaluated. This conclusion by a highly respected specialist Agency of the World Health Organisation (WHO) was reached after consideration of the universally available peer-reviewed information published in scientific journals by a group of specialists from several different countries, who were selected after careful consideration of their knowledge of the field and for their objectivity. These experts considered all of the carcinogenicity studies that have ever been conducted on MTBE and its primary, persistent metabolite, TBA. The final evaluation was based on "*inadequate evidence* in humans..." (there have been no epidemiological studies of MTBE) and "*limited evidence* in experimental animals for the carcinogenicity of MTBE." That this highly conservative organisation should have evaluated the experimental animal carcinogenicity information as providing "*limited evidence*" is remarkable. Tumour incidences were elevated in all studies, but they lacked any consistency except for Leydig cell adenomas, which were observed in two experiments in rats. The invited experts at the IARC evaluation clearly took note of some particular characteristics of the data-set that prevented them from arriving at a "*sufficient evidence*" evaluation. In my opinion and as described in more detail in McGregor (2006) the special characteristics were a lack of reproducibility in tumour types other than Leydig cell adenomas in rats (but not in mice) and the lack of human relevance for increased incidence in rats of Leydig cell adenomas. In addition, however, none of the elevated tumour incidences were for "cancers" (carcinomas); they were not malignancies but benign neoplasms described by pathologists as adenomas on the basis of their behaviour and microscopic morphology.

The EU (2002) evaluation of the carcinogenicity data for MTBE concluded as follows:

"MTBE produces tumours in mice and rats at doses ≥ 3,000 ppm after inhalation exposure. Tumours have been reported in rats at oral doses ≥ 250 mg/kg. There is no evidence of a direct genotoxic mode of action. Therefore, respiratory NOAEC of 400 ppm and oral LOAEL of 250 mg/kg are derived. There are indications of carcinogenicity in two species. However the treatment relation of the occurred tumours is equivocal in some studies (mouse adenoma) and the relevance of the mode of action is questionable in others (Leydig cell). Moreover, the tumours appear mostly at very high and systemically toxic doses, and MTBE is not genotoxic *in vitro* or *in vivo*. On the other hand, the human relevance of the testicular interstitial adenomas observed in rats on two separate rat strains cannot be neglected. In addition, certain uncertainty remains as to the significance of the lymphatic tumours found, in the light of the limitations of the study and inadequate reporting. The rapporteur considers MTBE as a borderline case between nonclassification and Carc. Cat. 3."

Carcinogen Category 3, I might point out, is reserved for substances that have shown some carcinogenic properties in rodents but the mode of action is considered *not* to be genotoxicity. It is in any case clear that the risk assessors are uncertain

whether MTBE is a carcinogen or not, on the basis of the evidence available to them. There are no carcinogenicity assays available to Dr. Burns in addition to those reviewed in IARC (1999) and EU (2002).

It is noted that neither of these substantial evaluations (IARC, 1999; EU, 2002) is mentioned by Dr. Burns, although she must surely have been aware of them, since they are both cited in McGregor (2006), a reference that is quoted by Dr. Burns. This demonstrates a sad selectivity in the formulation of Dr. Burns' opinions, particularly as she has found it so easy to cite papers published in a journal that is almost as inaccessible as the organisation that produces it (European Journal of Oncology, published by the European Ramazzini Foundation).

Dr. Burns appears to rely largely on the life-time study in Sprague-Dawley rats conducted by Belpoggi et al. (1997). This is the only reference to the study cited, but there was a series of publications of the same study (Belpoggi et al., 1995, 1997 and 1998; Maltoni et al., 1999) conducted according to the normal protocol of the laboratory, the European Ramazzini Foundation (ERF), which permits the rodents to die "naturally" rather than imposing a finite duration on the experiment. This is very unusual and introduces problems in the analysis of data that are severely compounded by the reluctance of the laboratory to submit to a proper and thorough review. Such inspections are required under the Good Laboratory Practice (GLP) regulations that have been in place since the late 1970s for all critical studies upon which regulatory decisions depend. Although GLP procedures were first set in place by the US Food and Drug Administration (FDA), they were rapidly adopted by other countries and organisations and were adopted internationally by the Organisation for Economic Cooperation and Development (OECD) in 1981. Additionally, it is common practice (although not universal) for a pathology review to take place in which pathologists involved with a study and pathologists who have not been involved with it come together to discuss and resolve problems and differences in border line diagnoses. The ERF has always resisted these cooperative practices, consequently generating suspicion and earning a reputation for secrecy. Quite recently, however, the ERF has transferred final data (but not histological specimens or note books, computer files, etc.) to the National Institute of Environmental Health Sciences (NIEHS), so they may enter the final information in their electronic database in North Carolina. This has been done for MTBE, which is now available for anyone one see. While this might appear to be an improvement on the previous situation, it is only rendering an appearance of legitimacy to a situation that was otherwise dubious.

The technique of exposing rats for two years and then maintaining them until they die of "natural causes" is said to increase the sensitivity of the carcinogenicity experiment in comparison with the more usual method in which the experiment is terminated after two years. It is not clear, however, that any direct comparison of these protocols has been undertaken. The life-time protocol can cause difficulties for the statistical analysis of tumours, because increases in tumour incidence are normally age-related and the majority of the tumours arise in aging animals irrespective of whether they are from treated or control groups. The likelihood of between group differences in survival is always present with such an indeterminate duration study design, no matter how carefully the doses are chosen on the basis of preliminary studies. Despite this drawback, it is recognised that the ERF laboratory is a rich source of carcinogenicity data and may be compared in this respect with the US National Toxicology Program, but the extent to which the data are comparable is largely unknown. On one of the rare occasions when microscope slides from an ERF

study were shared with external pathologists for examination (a carcinogenicity study of aspartame, from which a small number of slides selected by ERF were reviewed by an NTP Pathology Working Group), the conclusion reached by the Pathology Working Group was that the ERF study pathologists appeared to apply classifications different from those of the NTP pathologists and that their were problems arising as a result of (probably) chronic respiratory disease within the rats on the experiment (Hailey, 2001; Hailey, 2004; EFSA, 2006). The NTP pathologists also noted that the "peer review" procedure adopted was not according to their normal peer review protocol, but even this limited review identified that the evaluation criteria are different at ERF compared with NTP. It has long been recognised that interaction between pathologists is essential in order to reach or maintain a common understanding in the diagnosis of lesions (e.g., Amato and Lagakos, 1988). Finally, the ERF practice of providing multiple publications of the same study can lead to confusion because the different publications sometimes describe different results. When this happens, it is not clear which publication contains the definitive numbers and diagnoses, a feature that tends to undermine confidence in the data reported. For example, the Bundesinstitut für Risikobewertung point out (http://www.bfr.bund.de/cm/238/assessment_of_the_carcinogenicity_of_formaldehyde.pdf) that there had been a highly significant change in the evaluation of the histology in serial publications describing a single study of formaldehyde conducted by ERF (Soffritti et al., 1989; Soffritti et al., 2002a) (almost a doubling of the incidence of leukaemia in most treated groups of rats). In the case of MTBE, the findings for interstitial cell (Leydig cell) adenomas that had been consistently reported on three occasions (Belpoggi et al., 1995, 1997 and 1998) were differently reported in a fourth publication (Maltoni et al., 1999). Thus, there is ample basis for uncertainty regarding the ERF laboratory experiments in general.

Lymphoreticular neoplasm in rats.

As for the MTBE experiment in particular the most frequently found neoplasm in both the 250 and 1000 mg/kg body weight gavage MTBE exposure groups was lymphoimmunoblastic lymphoma, with a proportion of >85% of the combined incidence localised in the lungs. Leukaemia and lymphomas should usually be expected to arise in lymphoid tissue of some kind. They may subsequently move to other tissues, but there should never be an instance where the frequency of these neoplasms is greater in any single, non-lymphoid tissue within a group of rats than in lymphoid tissues. The authors presumed that they arose from peribronchial and perivascular lymphoid tissue (Belpoggi et al., 1998). Such neoplasms have been known to arise from the increased amount of lymphoid tissue in the lungs due to chronic pulmonary inflammation (Innes et al., 1967; Nelson, 1967; Swaen and van Heerde, 1973). The significance of chronic inflammatory changes in the lungs for the occurrence of pulmonary haemolymphoreticular dysplasias and neoplasias was not addressed in the Belpoggi et al. study, and this cannot be done on the basis of the publications because of the lack of detailed description of lung pathology. Nelson (1967) showed that the elimination of chronic respiratory disease from rats could reduce the incidence of lymphosarcomas almost to zero. Sinkeldam et al. (1991) found a high incidence of lymphoreticular tumours of the peribronchial tissue in high-dose male Wistar rats treated with acesulfame-K. Upon retesting in the same strain, but now cleaned and bred under specific-pathogen-free conditions, chronic respiratory disease was absent and no lymphoreticular tumours were seen. Thus, the tumours found in the first experiment appeared to be unrelated to treatment with acesulfame-

K, but were associated with the occurrence of peribronchial and perivascular lymphoid accumulations in response to chronic inflammation of the lungs (Feron et al., 1990).

An interesting difference between the studies with Sprague-Dawley and F344 rats is that the haematopoietic neoplasms involved are quite different. Those of Sprague-Dawley rats are B-cell derived, while the common haematopoietic neoplasm in F344 rats are non-B-, non-T-cell derived. These spontaneous neoplasms of F344 rats arise in spleen. B-cell neoplasms were not increased except in females of the Sprague-Dawley rat experiment. No effect on any haematopoietic neoplasms has been found in CD-1 mice treated with MTBE or in B6C3F1 mice treated with TBA or methanol. Thus the response described in female rats by Belpoggi et al. has not been described in male rats of the same experiment, in rats of either sex in another study with MTBE (Chun et al., 1992), in a mouse study with MTBE (Burleigh-Flayer et al., 1992), or in either rats or mice treated with TBA (NTP, 1995), the more persistent of the primary metabolite of MTBE. The only support for their result is the drinking-water study of methanol conducted at the same laboratory (Soffritti et al., 2002b), but even this support is not found in another carcinogenicity study of methanol (NEDO, 1987).

In conclusion, an effect of MTBE on the incidence of lymphoreticular neoplasms has not been clearly demonstrated in the gavage administration experiment in which an increased incidence was observed in female rats, but not in male rats. There is serious doubt regarding the pathogenesis of these neoplasms. Even if the response was truly to MTBE or its metabolite formaldehyde, it could be a Cmax effect at which normal defence mechanisms had been overwhelmed; this would be an extremely unlikely occurrence during human exposure.

Leydig cell tumour in rats.

Elevated incidences of Leydig cell tumours (i.e., biochemically important, non-germ cells in the testes) were found in rats in two studies, one with F344 rats (Chun et al., 1992; Bird et al., 1997) and one with SD rats (Belpoggi et al., 1997). It is my opinion that these tumours in rats have no significance for human health (see McGregor, 2006 for discussion of this topic); furthermore, it is not clear that the elevated incidence was actually reproducible. In the inhalation study with F344 rats (Chun et al. (1992; Bird et al., 1997) the statistically significant increase in Leydig cell adenoma incidence in the 3000 ppm dose and not the 8000 ppm dose group was due to two factors. Firstly, chronic progressive nephropathy (CPN) was a significant cause of death among male rats in the high dose group, thereby reducing the chance of them developing Leydig cell adenomas; and, secondly, the incidence of Leydig cell adenomas in the control group was very low (64%) when compared with the historical incidence of this tumour in earlier experiments from the same laboratory (86% to 91%). No reason for this departure from the expected incidence has emerged. The accumulated historical control group incidences in this laboratory were very similar to the 74% to 98% range found in US NTP laboratories (Haseman et al., 1998).

Leydig cell tumours do not usually occur in less than one year in rats and they are strongly associated with advancing age. Thus, the statistically significant increased incidence in the high dose group of one study (Belpoggi et al., 1997) could be a consequence of the paradoxically reduced survival in the control group. Indeed, the survival difference is particularly surprising because excessive toxicity early in the study dictated that dosing with MTBE should be reduced from daily administration to just four days per week. It is also surprising that there should have been no mention

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